DETERMINATION OF POLYNUCLEAR AROMATIC HYDROCARBONS IN FISH

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Ontario
Ministry of the Environment

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ABSTRACT

Three methods were evaluated for the extraction of polynuclear aromatic hydrocarbons (PAH) from fish tissue. The extraction techniques were geared towards high performance liquid chromatography (HPLC) using ultraviolet and fluorescence detectors. Using the liquid-liquid partition method, fish were saponified, extracted with cyclohexane, and the extracts partitioned into dimethylformamide-water-cyclohexane. This extraction procedure provided the best recoveries of spiked PAH. Ten carp and ten pike were sampled from Hamilton Harbour, and the Rouge River in Detroit, Michigan, and four PAH were identified and quantitated in fish at the ng/kg range.

TABLE OF CONTENTS

	Page
TITLE PAGE	i
ABSTRACT	ii
TABLE OF CONTENTS	iii
LIST OF FIGURES	iv
ACKNOWLEDGEMENTS	v
1. INTRODUCTION	1
2. EXPERIMENTAL	2
2.1 Apparatus	2
2.2 Reagents	2
2.3 HPLC Operating Conditions	3
2.4 Fish Preparation	3
2.5 Extraction Methods	4
2.5.1 Method 1	4
2.5.2 Method 2	5
2.5.3 Method 3	6
3. RESULTS AND DISCUSSION	7
4. REFERENCES	18
5. APPENDIX	20

LIST OF FIGURES

Figure		Page
1	Method 1 Flow Diagram	8
2	High Performance Liquid Chromatogram of Fish Glyceride-Type Lipids on a Reverse Phase Column	11
3	Method 2 Flow Diagram	13
4	Method 3 Flow Diagram	15
5	High Performance Liquid Chromatogram of a Fish Extract Prepared Using Method 3	16

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The authors are also grateful to O. Meresz, J. N. Bishop and G. C. Ronan for their interest and encouragement.

1. INTRODUCTION

Polynuclear aromatic hydrocarbons (PAH) originating from a number of sources occur widely in the environment including freshwater and marine sediments[1-3], and in biota. As many of these compounds are known to be carcinogenic[4], there has been considerable interest in developing reliable analytical methods for low levels of PAH in biological samples.

Presently, a variety of analytical techniques exist for the determination of PAH in marine biota[5-9]. To remove PAH from the tissue matrix, the methods generally employ soxlet extraction, alcoholic and/or aqueous caustic digestion followed by organic solvent extraction, or homogenization in the presence of an organic solvent.

In the present study, extraction techniques were evaluated for the determination of PAH. High performance liquid chromatography (HPLC) using a combination of ultraviolet and fluorescence detectors[10] was used for the separation and detection of PAH components. The extraction techniques were geared towards this detection system which is relatively specific for PAH, and minimizes interference from other biotic hydrocarbons or organic pollutants.

2. EXPERIMENTAL

2.1 Apparatus

A Varian Model 8500 Liquid Chromatograph equipped for gradient elution and with a Schoeffel FS-970 fluorescence detector and a Varian Varichrome UV/VIS detector (in series[10]) was used. The column used was a Vydac 201 TP (The Separation Group) reverse phase column.

2.2 Reagents

Spectrograde methanol was obtained from Fisher Scientific Co. Inc.; acetonitrile was obtained from Burdick and Jackson Inc.; cyclohexane (distilled-in-glass) was obtained from Caledon Laboratories Ltd.; "Florisil" (60-100 mesh), calcium chloride, and celite were obtained from BDH Chemicals; propylene carbonate was obtained from Canadian Laboratory Supplies; and alumina (neutral, 80-200 mesh) was obtained from Fisher Scientific Co. Ltd.

PAH standards used in this study were from the following sources: pyrene, benzo(e)pyrene (BeP), perylene(Per) benzo(g,h,i)perylene (BghiP) and coronene (Cor) from Aldrich Chemical Co.; benzo(a) pyrene (BaP) and dibenz(a,h)anthracene (DBahA) from Eastman Kodak Co.; chrysene and fluoranthene from J. T. Baker Chemical Co.; benzo(k)fluoranthene (BkF) from

J. L. Monkman, Environment Canada; o-phenylenepyrene (OPP) and anthanthrene (AA) were obtained from F. I. Onuska, CCIW, Burlington, Ontario. The structures for these PAH can be found in Appendix 1. Organic-free water was prepared by passing distilled water through a "Millipore Super Q" system.

2.3 HPLC Operating Conditions

The eluent was a mixture of acetonitrile (75%) and water (25%, V/V) applied at a pressure of 105 kg/cm^2 and a flow rate of 1.0 mL/min. The analyses of the synthetic mixture of PAH and those from environmental samples were both performed under these conditions at ambient temperature. When analyses were carried out using detectors at set wavelengths, the UV absorbance was measured at 254 nm, and the fluorescence λexcitation = 365 nm, and λemission >415 nm.

In analyses where the wavelengths were varied, the UV detector was monitored at 240 nm (for fluoranthene and pyrene), and 295 nm (for BkF, BaP, DBahA, and BghiP). The fluorescence detector was set at λexcitation = 254 nm, λemission >370 nm for perylene, BkF and BaP. The baselines were adjusted as necessary after changing detector wavelengths.

2.4 Fish Preparation

All mucous was washed off the fish, which were then cut posteriorly along the dorsal surface with a stainless steel

knife. The epaxial musculature (the muscle above the lateral line) was stripped from the skin and about 50 g was placed into a stainless steel cup, and the tissue was ground for two minutes using a Virtis Homogenizer. The homogenized tissue was then transferred to an aluminum foil cup, and covered with aluminum foil.

2.5 Extraction Methods

2.5.1 Method 1

To 50 g of ground fish was added 50 mL of hexane-acetone (4:1) in a stainless steel container and the contents were thoroughly mixed (extracted) in a Virtis Homogenizer for ten minutes. The solvent mixture was decanted, another 50 mL hexane-acetone was added to the residue, and the extraction procedure was repeated two more times. The combined extracts were carefully concentrated to dryness and taken up in a small volume of cyclohexane. This extract was then chromatographed on 100 g of Alumina (activity IV, deactivated with 10% water), using cyclohexane (300 mL). The eluate was collected and evaporated to dryness. The residue made up to 1.0 mL with acetonitrile-water (3:1), and was ready for HPLC analysis.

2.5.2 Method 2

To 50 g of ground fish was added 50 mL of chloroform in a stainless steel container and thoroughly mixed for ten minutes in a Virtis Homogenizer. Next, 25 g of sodium sulphate was added and the mixture homogenized, followed by 50 g of celite and homogenization once more. This mixture was transferred to a 500 mL round bottom flask and the chloroform removed by rotary evaporation.

The resulting solid was then packed into a column (4 cm I.D. x 40 cm) and eluted with 100 mL of propylene carbonate. The eluate was then transferred to a 1.0 L separatory funnel containing 500 mL of an aqueous 5% sodium hydroxide. This mixture was shaken vigorously, to hydrolyse the propylene carbonate to propylene glycol.

The alkaline solution thus obtained was extracted with four 50 mL portions of cyclohexane and the aqueous phase was then discarded. A mixture of 18 g of granular anhydrous calcium chloride and 8 g of celite was then added to the separatory funnel containing the combined eluants and shaken. The cyclohexane extract was then drained from the separatory funnel, concentrated to approximately 0.5 mL, and chromatographed on 2.0 g of deactivated florisil, eluting with 50 mL of cyclohexane. The eluate was concentrated to dryness and made up to 1.0 mL with acetonitrile-water (3:1), ready for HPLC analysis.

2.5.3 Method 3

To 50 g of ground fish in a 500 mL round bottom flask was added 100 mL of a 2N solution of potassium hydroxide in methanol-water (9:1), and this mixutre was refluxed for twelve hours.

The saponified mixture, while still warm, was transferred to a 1.0 L separatory funnel, and the flask rinsed with 200 mL methanol-water (4:1). This mixture was extracted with three 100 mL portions of cyclohexane, and the basic fraction was discarded. The cyclohexane extract was then washed two times with 100 mL of methanol-water (1:1), and two times with 100 mL of water, successively, then concentrated to 60 mL by rotary evaporation.

The concentrate was then extracted two times with 60 mL portions of a dimethylformamide (DMF)-water (9:1) solution, and the cyclohexane was discarded. Next, 120 mL of water was added to the DMF-water solution and extracted two times with 60 mL of cyclohexane. The DMF-water solution was then discarded.

Finally, the cyclohexane extract was washed two times with 20 mL of brine and concentrated to approximately 0.5 mL. This extract was chromatographed on 2.0 g of deactivated florisil (deactivated by the addition of 15% water), eluting with 50 mL of cyclohexane. The eluate was concentrated

by rotary evaporator to approximately 0.5 mL, then carefully evaporated to dryness under a stream of nitrogen. The extract was then made up to 1.0 mL with acetonitrile-water (3:1) and was ready for HPLC analysis.

3. RESULTS AND DISCUSSION

There were certain criteria that had to be met in establishing a procedure for the extraction of PAH from fish tissue. The method had to be fast and relatively simple, and provide acceptable recoveries. It was also desirable to separate the PAH from fish protein, lipids and other hydrocarbons that would interfere with the PAH analysis.

Three methods that are designed to preferentially separate PAH from a protein and lipid matrix were evaluated. Northern pike (Esox lucius) from Bolster Lake in the Thunder Bay region of Northwestern Ontario were used in the recovery studies. The first procedure (Method 1, above) evaluated was a method by Telling[11], which was a clean-up technique based on a single column of deactivated alumina, and was designed for the determination of organohalide insecticide residues. The ground fish tissue was homogenized in the presence of a hexane/acetone solvent system. The flow diagram is presented in Figure 1. The concentrated organic residue was carefully chromatographed on a deactivated alumina column. Recoveries of spiked PAH were poor (see Table 1) as determined by the

FIGURE 1

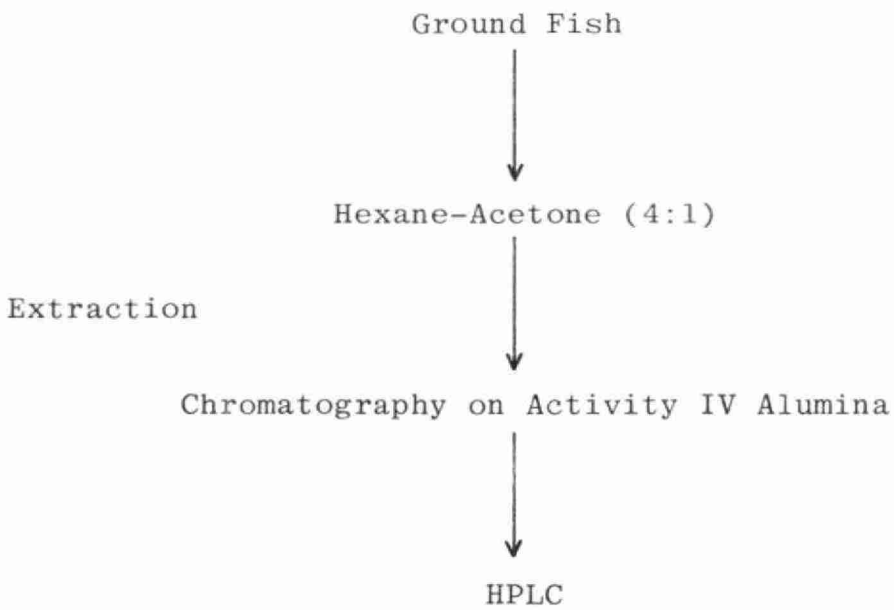

Ground Fish
Hexane-Acetone (4:1)
Extraction
Chromatography on Activity IV Alumina
HPLC

TABLE 1

Recoveries Studies of Spiked PAH in Fish

*Method 1 (Telling)

PAH	% Recovery
Perylene	15
Benzo(k)fluoranthene	26
Benzo(a)pyrene	8
Coronene	30

Mean Recovery.....19.8%
Standard Deviation.....10.1

*Method 2 (Potthast)

PAH	% Recovery
Perylene	19
Benzo(k)fluoranthene	42
Benzo(a)pyrene	67
Coronene	53

Mean Recovery.....45.3%
Standard Deviation.....20.3

*Method 3 (Grimmer)

PAH	% Recovery
Perylene	74, 97
Benzo(k)fluoranthene	79, 97
Benzo(a)pyrene	83, 105
Coronene	75, 93

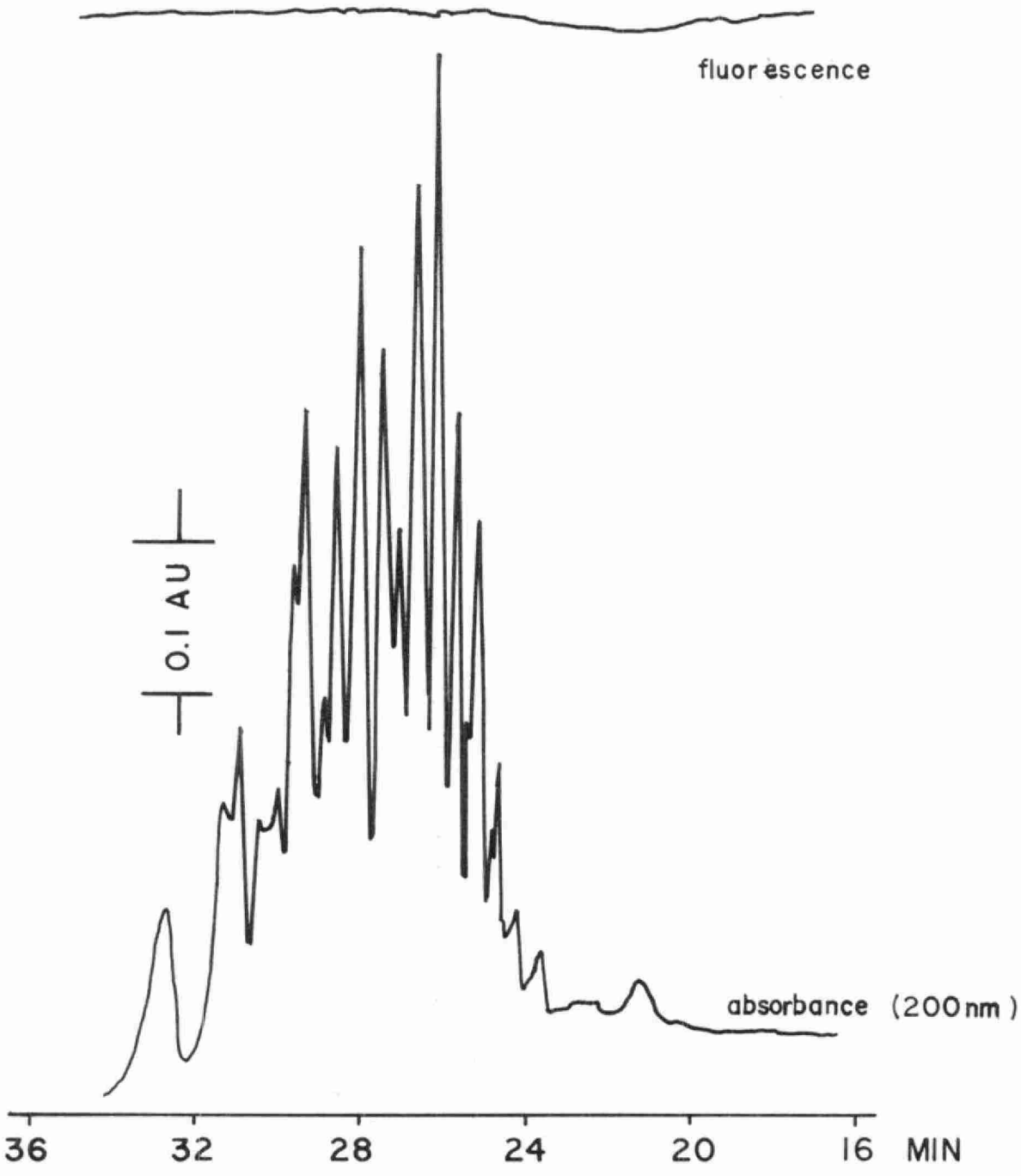
Mean Recovery.....87.9%
Standard Deviation.....11.6

* Spike concentration = 100 ng/kg wet weight fillet

fluorescence response in HPLC. Interestingly enough, well resolved peaks in the chromatogram were detected by ultraviolet response (λmax 200 nm) with little fluorescence response. These peaks had much longer retention times than the PAH in question. The HPLC fraction where these peaks appeared was collected, concentrated, and analyzed by infrared spectroscopy. Strong absorbances at 5.74 um, and 8.58 um indicated the presence of an ester, while the absorbance at 8.58 um could also be accounted for by a phosphorus-oxygen bond. Strong absorbances from 3.3 - 3.5 um, and weaker absorbances at 6.06 and 6.85 um indicated the presence of alkane and alkene hydrocarbon chromophores. A weak absorption at 13.85 um indicated the presence of an alkane moiety with a chain length of greater than four carbon atoms. It was surmised that these compounds were glyceride-type lipids. In fact, the resolution by HPLC of these compounds was far superior to anything reported in the literature[12] (see Figure 2), and may warrant further investigation if the need arises.

The second procedure evaluated (Method 2) was a technique by Potthast[13] for the rapid isolation of PAH from smoked meat products. The procedure is based on the observations that the PAH are apparently more soluble in propylene carbonate than in most other organic solvents, and that extraction with this solvent yields a fat-free extract.

FIGURE 2



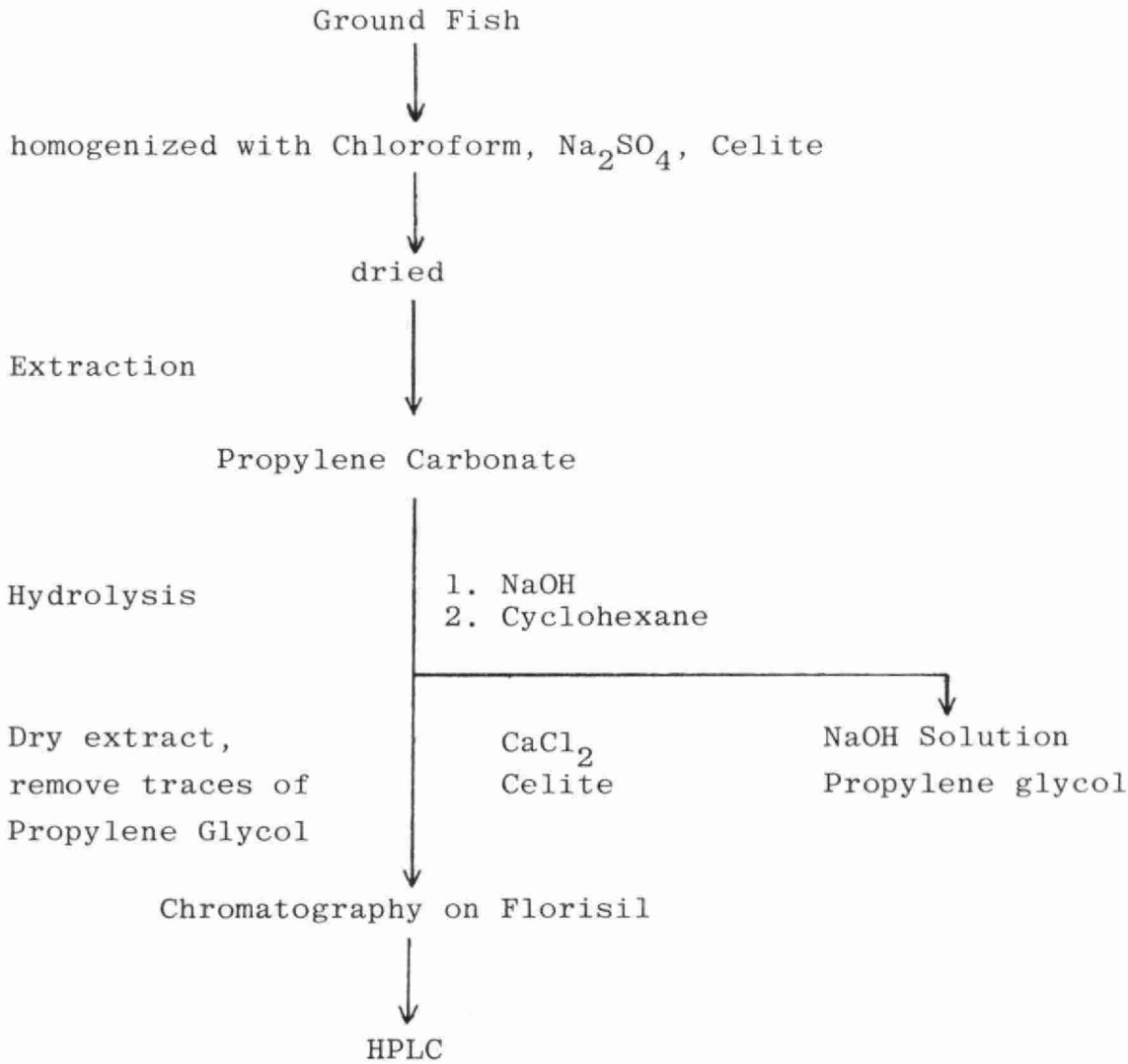
The ground fish tissue was homogenized in the presence of chloroform (see Figure 3). Next, sodium sulphate and celite were added to the mixture. The solvent was removed under reduced pressure and the residual material transferred to a column and eluted with propylene carbonate. Propylene carbonate is a high boiling material (b.p. 240^{o}), and not a suitable solvent to use in either GC or HPLC analysis. Thus, the PAH had to be extracted from this solvent. This was accomplished by an aqueous saponification of the propylene carbonate, then extraction of the aqueous phase with cyclohexane.

Recovery of PAH using this method was only fair (see Table 1). This technique was quite time consuming; the final extract was not fat free; the column that was eluted channelled very badly. This channelling may have been caused by the rather non-homogenous nature of the material when the column was packed.

Both of the aforementioned methods offered only limited advantages, such as reduction in the lipid level, over conventional soxlet extraction. However, the disadvantages far outweighed the advantages.

It appeared that saponification of the fish tissue would be an appropriate approach. This would hydrolyze lipids to the corresponding aliphatic acids, glycols and phosphates which could be readily removed. Further, there would be

FIGURE 3


Ground Fish
homogenized with Chloroform, Na_2SO_4, Celite
dried
Extraction
Propylene Carbonate
Hydrolysis
1. NaOH
2. Cyclohexane
Dry extract,
remove traces of
Propylene Glycol
$CaCl_2$
Celite
NaOH Solution
Propylene glycol
Chromatography on Florisil
HPLC

complete destruction of the fish protein matrix which had been shown to adsorb PAH to some degree[14].

The method published by Grimmer[14], and modified somewhat for our purposes (Method 3), proved to be the most suitable and successful. The flow diagram is illustrated in Figure 4. The ground fish was saponified with methanolic potassium hydroxide, than extracted with cyclohexane. The cyclohexane phase was extracted with methanol-water, then partitioned into a dimethylformamide (DMF)-water solution, and extracted with cyclohexane, discarding the cyclohexane extracts. Water was added to the DMF-water solution, and this was extracted with cyclohexane, discarding the DMF-water solution. After filtration of the cyclohexane extracts through Florisil, and concentration, the extract was ready for analysis.

This extraction (and clean-up) technique gave the best recovery (87.9%) of spiked PAH (see Table 1). The extract obtained by this method was quite suitable for analysis by HPLC (using fluorescence detection). A typical HPLC analysis of a fish extract is illustrated in Figure 5.

Gas chromatographic analysis indicated the presence of a large number of peaks, presumably cyclic and acyclic alkanes and isoprenoid hydrocarbons. These extraneous peaks were considerably reduced by repartitioning the cyclohexane extract into DMF-water, and repeating the last phase of the partition method.

FIGURE 4

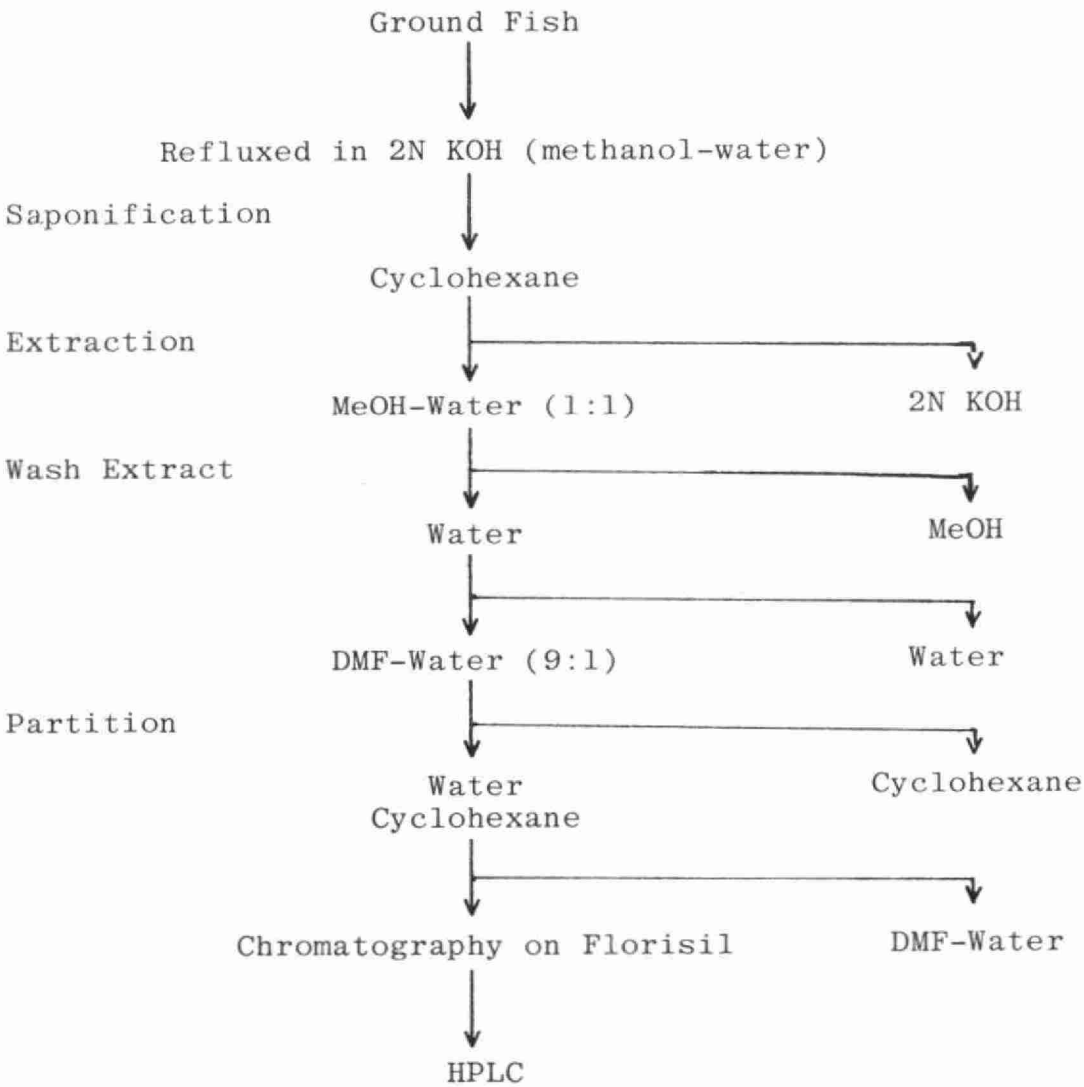
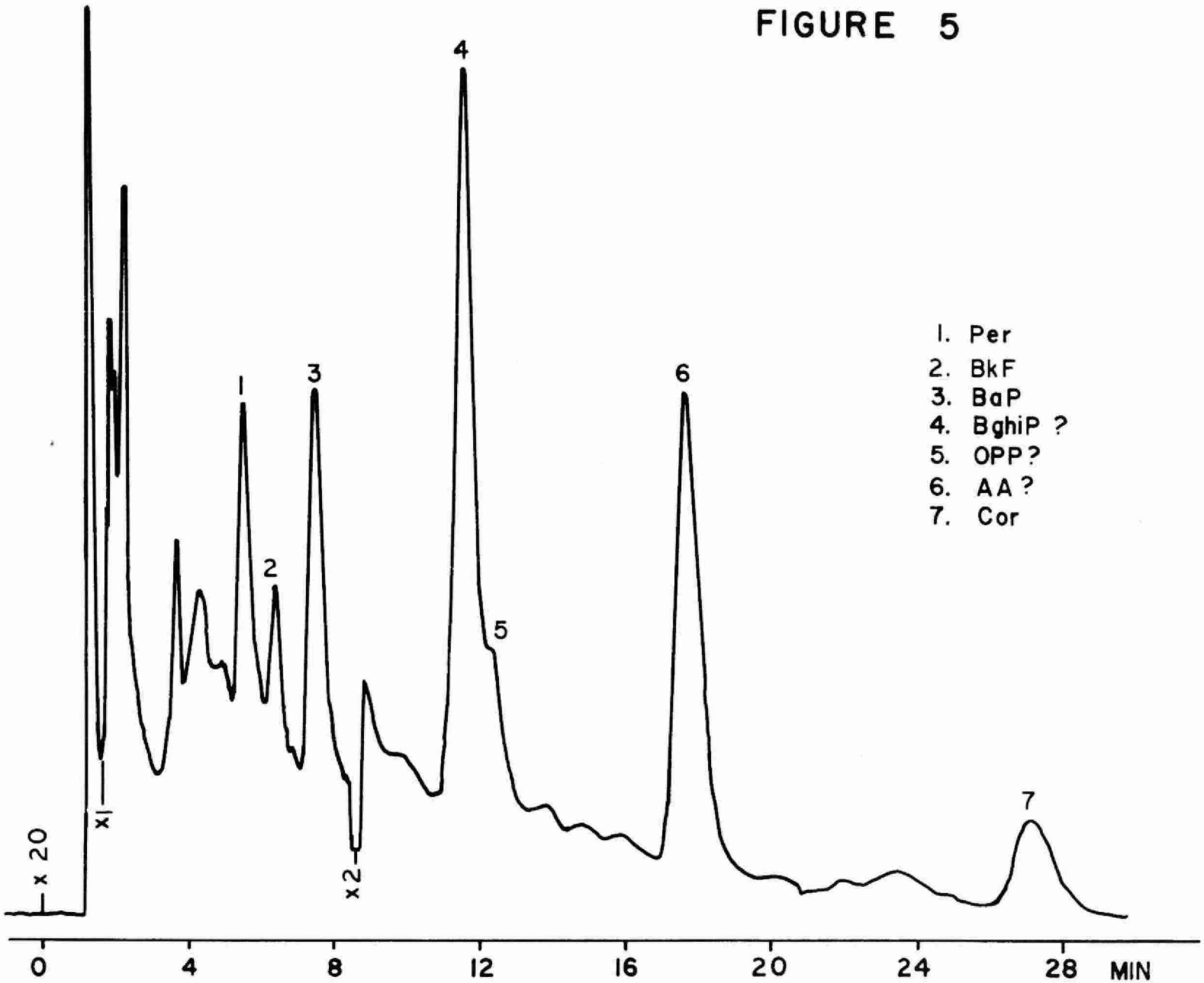

Ground Fish
Refluxed in 2N KOH (methanol-water)
Saponification
Cyclohexane
Extraction
MeOH-Water (1:1)
2N KOH
Wash Extract
Water
MeOH
DMF-Water (9:1)
Water
Partition
Water
Cyclohexane
Cyclohexane
Chromatography on Florisil
DMF-Water
HPLC

FIGURE 5


1. Per
2. BkF
3. BaP
4. BghiP ?
5. OPP?
6. AA ?
7. Cor
x 20
x1
x2
0
4
8
12
16
20
24
28
MIN

In several instances, serious emulsion problems arose. In almost all cases, it was possible to break these eumlsions by the addition of sodium chloride.

As part of a joint project with the Wildlife Toxicology Division of the Canadian Wildlife Service, fish from two centres having heavy industry and a high density of population were analyzed for PAH. Ten carp (Cyprinus carpis) and ten northern pike (Esox lucius) were sampled from Hamilton Harbour - Hamilton, Ontario and an equivalent sample was taken from the confluence of the Rouge and Detroit Rivers - Detroit, Michigan. It was jointly agreed by the project participants that only those PAH that were positively identified by gas chromatographic/mass spectrometric analysis would be quantitated.

The levels of PAH detected in the fish were extremely low (parts per trillion). The range of contamination observed was from the detection limit of 5 ng/kg wet weight fillet for perylene, benzo(k)fluoranthene and benzo(a)pyrene and 20 ng/kg for coronene to 400 ng/kg. These values are contained in Appendix 2, Table 1. The Appendix contains a detailed abstract of the presentation "Incidence of Polynuclear Aromatic Hydrocarbons in Fish Near Two Industrial Centres" presented at the International Symposium on the Analysis of Hydrocarbons and Halogenated Hydrocarbons, May 1978, in Hamilton, Ontario.

4. REFERENCES

1. M. Strosher and G. W. Hodgson, Occurrence of PAH's in Sediments and Water in the Great Lakes, Federal Government Contract 01-GR. KW 412-2-1052, (1973).

2. W. Giger and M. Blumer, Anal. Chem., 46, 1663, (1974).

3. M. Blummer and W. W. Youngblood, Science 188, 53, (1975).

4. P. Shubik and J. L. Hartwell, Survey of Compounds Which Have Been Tested For Carcinogenic Activity, Public Health Service Publication, No. 149, Suppl. II. (1969).

5. H. J. Cahnmann and M. Kutatsune, Anal. Chem., 29, 1312, (1957).

6. J. S. Warner, Anal. Chem., 48, 578, (1976).

7. B. P. Dunn, Environ. Sci. & Technol., 10, 1019, (1976).

8. F. I. Onuska, A. W. Wolkoff, M. E. Comba, R. H. Larose, M. Novotny and M. L. Lee, Anal. Letters, 9, 451, (1976).

9. S. N. Chesler, B. H. Gump, H. S. Hertz, W. E. May and S. A. Wise, Anal. Chem., 50, 805, (1978).

10. R. D. Smillie, D. T. Wang and O. Meresz, J. Environ. Sci. Health A13, 47, (1978).

11. G. M. Telling, D. J. Sissons and H. W. Brinkman, J. Chromatography, 137, 405, (1977).

12. N. A. Parris, J. Chromatography, 149, 615, (1978).

13. K. Potthast and G. Eigner, J. Chromatography, 103, 173, (1975).

14. G. Grimmer and H. Bohnke, J.A.O.A.C., 58, 725, (1975).

APPENDIX

The Abstract of the presentation "Incidence of Polynuclear Aromatic Hydrocarbons in Fish Near Two Industrial Centres", presented at the International Symposium on the Analysis of Hydrocarbons and Halogenated Hydrocarbons, May 1978, in Hamilton, Ontario.

Incidence of Polynuclear Aromatic Hydrocarbons in Fish Near Two Industrial Centres

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F.I. Onuska and M.E. Comba, Analytical Methods Research Section, Canada Centre for Inland Waters, Fisheries and Environment Canada, Burlington, Ontario

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Polynuclear aromatic hydrocarbons (PAH) have been found to occur in a variety of freshwater and marine sediments and associated benthic fauna, terrestrial soils, and in the atmosphere, particularly in those areas proximate to industrialized centres (Grimmer and Bohuke, 1975, Lunde and Bjorseth, 1977, Kawakami and Nishimua, 1976). Recent studies conducted by the Canadian Wildlife Service have shown a broad range of PAH to be present in the body lipid of adult Herring Gulls taken from the Kingston area (Hallett et al., 1977a). Other studies have shown them to be prevalent in Lake Ontario sediments (Strosher and Hodgson, 1976). Herring Gulls are fish-eating birds and have been shown to bioaccumulate high residue levels of the more persistent organochlorine pollutants from their diet. Therefore, ten carp Cyprinus carpio and ten pike Esox lucius were sampled from Hamilton Harbour, Hamilton, Ontario and an equivalent sample taken from the confluence of the Rouge and Detroit Rivers at Detroit, Michigan - two centres having heavy industry and a high density of population.

Fluorescence detection is relatively specific for multi-ringed aromatic hydrocarbons and minimizes interference from other types of biogenic hydrocarbons or organic pollutants (Smillie et al., 1978).

Therefore, this technique was chosen for accurate quantitation (Table 1). However, fluorescence detection L.C. would not resolve the multitude of PAH found at trace levels in the fish samples. Consequently, high resolution capillary GC/MS was also utilized. This resulted in the identification of twenty more PAH (Table 2). Electron impact spectra were obtained on a Varian Mat 311A mass spectrometer, interfaced to a Varian 2700 gas chromatograph via open coupling. Sample separation was achieved by employing 20M SP-2100 and OV-17 capillary columns.

Additional confirmation of PAH was performed using computer controlled multiple ion selection (MIS) in the accelerating voltage alteration (ava) mode. Twenty-one individual masses were monitored over a gas chromatographic run with each preselected mass prefocused to a standard PAH. After initialization the data system automatically scans each data block and provides an integrated listing with a corresponding time scale. Bar graph plots of each monitored mass are then obtained for comparison to a standard reference.

The GC/MS technique is extremely expensive and time consuming, and accurate quantitations were not performed using Multiple Ion Scanning at this time. This method is presently being developed and preliminary quantitative data were in agreement with the L.C. fluorescence levels. Since the detection limit for these fish samples was approximately 10 ng for each compound, the total amount of PAH is somewhat higher than the fluorescnece L.C. data.

Carcinogenic PAH identified in the Great Lakes fish included benz-(a)-pyrene, and dibenz-(a,h)-anthracene. Suspected carcinogens include 1,2-benzofluorene, 2,3-benzofluorene, chrysene, perylene, pyrene, phenanthrene, and methyl phenanthrenes.

The levles of PAH detected in fish are extremely low (ng/kg to µg/kg wet weight fillet). Their detection reflects the advanced technology utilized for environmental analysis. Levels in Herring Gulls

are also extremely low (Hallettet al., 1977a) relative to organochlorine residues, which range as high as 3000 mg/kg body lipid for PCB (Hallett et al., 1977b). The actual hazard of these PAH to Herring Gulls and fish is presently unknown.

Analysis of Herring Gulls and eggs has been used successfully as an "early warning system" for hazardous chemicals which bioaccumulate in the food web of the Great Lakes (Gilman et al., 1977). The presence of PAH both in Great Lakes fish and in Herring Gulls indicates that it is possible for these compounds to accumulate via the food chain. PAH probably metabolize faster than chlorinated aromatics such as PCB, and therefore PAH would not bioaccumulate to the same extent. However, the evidence definitely indicates that Great Lakes fish and Herring Gulls are being exposed to PAH, including known carcinogens.

References

Grimmer, G. and H. Bohuke. 1975. Cancer Letters 1:75-84.

Gilman, A.P., D.B. Peakall, D.J. Hallett, G.A. Fox, and R.J. Norstrom. 1978. Proc. Symp. Environ. Pollut., Storrs Conn., Nat. Acad. Sci.; (in press).

Hallett, D.J., R.J. Norstrom, F.I. Onuska, and M.E. Comba, 1977a. Proc. 2nd. Intl. Symp. Glass Capillary Chromatorgraph, Hindelang, Germany, May 1977. pp. 115-125.

Hallett, D.J., R.J. Norstrom, F.I. Onuska, and M.E. Comba. 1977b. in Fate of Pesticides in the Large Animal. Academic Press, pp. 183-191.

Kawakami, Y. and H. Nishimura. 1976. J. of Oceanograph. Sci. Japan. 32:175-181.

Lunde, G. and A. Bjorseth. 1977. Nature, 268:518-520.

Smillie, R.D., D.T. Wang, and O. Meresz. 1978. J. Environ. Sci. Health, A1 3(1):47-59.

Strosher, M.T. and G.W. Hodgson. 1975. Water Quality Parameters, ASTM STP 573. American Society for Testing Materials: 259-270.

Table 1

Quantitation of PAH in Great Lakes Fish by
Fluorescence Detection using Liquid Chromatography

Fish	Polynuclear Aromatic Hydrocarbons ng/kg fresh weight fillet			
	Perylene	Benzo-(k)-fluoranthene	Benzo-(a)-pyrene	Coronene
a) Hamilton Harbour carp				
1	46	8	108	300
2	140	40	160	360
3	26	12	96	210
4	160	40	200	400
5	40	16	108	220
6	74	12	160	60
7	40	8	144	300
8	142	68	268	320
9	nd	nd	nd	20
b) Hamilton Harbour pike				
1	90	48	154	240
2	64	32	128	200
3	40	12	70	200
4	32	10	54	220
5	58	20	74	200
6	40	12	64	170
7	34	8	60	152
8	40	12	54	140
9	nd	nd	nd	nd
10	20	12	34	100
c) Detroit carp				
1	16	10	40	80
2	nd	nd	nd	60
3	40	14	40	nd
4	26	10	40	40
5	nd	nd	nd	nd
6	nd	nd	nd	nd
7	nd	nd	nd	120
8	nd	nd	nd	80
9	nd	nd	nd	nd
10	nd	nd	nd	nd
d) Detroit pike				
1	34	26	40	20
2	20	14	14	40
3	18	8	34	40
4	20	8	20	44
5	68	26	128	290
6	18	10	24	40
7	20	6	30	30
8	nd	nd	nd	nd
9	46	24	70	120
10	52	26	100	120

nd = non detectable

detection limits 5 ng/kg perylene, benzo-(k)-fluoranthene, benzo-(a)-pyrene
20 ng/kg coronene

Table 2

Polynuclear Aromatic Hydrocarbons Identified
by Mass Spectrometry in Great Lakes Fish

PAH	Hamilton Harbour carp	Hamilton Harbour pike	Detroit River carp	Detroit River pike
1. naphthalene	x	x		x
2. 2-methyl naphthalene	x	x		x
3. 1-methyl naphthalene	x	x		x
4. biphenyl	x	x		x
5. acenaphthene		x		x
6. dimethyl naphthalene		x		x
7. fluorene		x		x
8. anthracene	x	x		x
9. phenanthrene	x	x		x
10. 1-phenyl naphthalene	x	x		x
11. 1-methyl phenanthrene	x	x		x
12. 1-methyl anthracene	x	x		x
13. 2-methyl anthracene	x	x		x
14. 2-methyl phenanthrene	x	x		x
15. 9-methyl anthracene				x
16. fluoranthrene	x	x		x
17. pyrene	x	x		x
18. 1, 2-benzofluorene		x		x
19. 2, 3-benzofluorene		x		x
20. chrysene	x	x		x
21. benzo-(a)-pyrene		x		x
22. perylene		x		x
23. dibenz-(a, h)-anthracene	x	x		x
24. coronene	x	x		x

x detected

Other compounds scanned for but not found include 4 methyl biphenyl, 3, 6 dimethyl phenanthrene, 9, 10 dimethyl anthracene, 1-methyl pyrene, 1, 1 binaphthyl, benzo-(e)-pyrene, 9, 10 diphenyl anthracene, ananthrene, benzo-(g, h, i)-perylene, picene, and dibenz pyrenes.